Nonesterified Fatty Acids and Hepatic Glucose Metabolism in the Conscious Dog

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We used tracer and arteriovenous difference techniques in conscious dogs to determine the effect of nonesterified fatty acids (NEFAs) on net hepatic glucose uptake (NHGU). The protocol included equilibration ([3-^{3}H]glucose), basal, and two experimental periods (−120 to −30, −30 to 0, 0–120 [period 1], and 120–240 min [period 2], respectively). During periods 1 and 2, somatostatin, basal intraportal insulin and glucagon, portal glucose (21.3 μmol · kg^{-1} · min^{-1}), peripheral glucose (to double the hepatic glucose load), and peripheral nicotinic acid (1.5 mg · kg^{-1} · min^{-1}) were infused. During period 2, saline (nicotinic acid [NA], n = 7), lipid emulsion (NA plus lipid emulsion [NAL], n = 8), or glycerol (NA plus glycerol [NAG], n = 3) was infused peripherally. During period 2, the NA and NAL groups differed (P < 0.05) in rates of NHGU (10.5 ± 2.08 and 4.7 ± 1.9 μmol · kg^{-1} · min^{-1}), respectively, endogenous glucose R_a (2.3 ± 1.4 and 10.6 ± 1.0 μmol · kg^{-1} · min^{-1}), net hepatic NEFA uptakes (0.1 ± 0.1 and 1.8 ± 0.2 μmol · kg^{-1} · min^{-1}), net hepatic β-hydroxybutyrate output (0.1 ± 0.0 and 0.4 ± 0.1 μmol · kg^{-1} · min^{-1}), and net hepatic lactate output (6.5 ± 1.7 vs. −2.3 ± 1.2 μmol · kg^{-1} · min^{-1}). Hepatic glucose uptake and release were 2.6 μmol · kg^{-1} · min^{-1} less and 3.5 μmol · kg^{-1} · min^{-1} greater, respectively, in the NAL than NA group (NS). The NAG group did not differ significantly from the NA group in any of the parameters listed above. In the presence of hyperglycemia and relative insulin deficiency, elevated NEFAs reduce NHGU by stimulating hepatic glucose release and suppressing hepatic glucose uptake. *Diabetes* 53:32–40, 2004

Elevated levels of nonesterified fatty acids (NEFAs) impair insulin-mediated suppression of endogenous glucose production under postabsorptive conditions (1–9). However, the relationship between NEFA concentrations and net splanchnic or hepatic glucose uptake (HGU) in the postprandial state is less clearly understood. NEFA concentrations and net hepatic or splanchnic uptake of NEFA fall during the postprandial period (10–12). Postprandial NEFA concentrations are higher in individuals with type 2 diabetes than nondiabetic individuals, even though their insulin concentrations are also higher (13,14). Normally, the liver extracts approximately one-third of a glucose load delivered enterally or into the portal vein (15–17), but net HGU (NHGU) is reduced in individuals with type 2 diabetes (13,18) and in those at risk of developing type 2 diabetes (19,20). Whether an elevation of NEFA concentrations might contribute to impaired postprandial HGU has not been established.

We hypothesized that the failure to suppress NEFA concentrations postprandially would reduce NHGU. To test this hypothesis, we studied conscious dogs under euinsulinemic-hyperglycemic conditions as a first step toward understanding the impact of elevated NEFA concentrations in conjunction with the relative insulin deficiency of type 2 diabetes.

RESEARCH DESIGN AND METHODS

Studies were carried out on conscious mongrel dogs of both sexes, fasted 42 h, and with a mean weight of 23.6 ± 0.9 kg. The 42-h fast was chosen because it is a time when hepatic glycogen has reached a stable minimum in both dogs and humans (21,22). Approximately 16 days before study, each dog underwent a laparotomy for placement of ultrasonic flow probes (Transonic Systems, Ithaca, NY) around the portal vein and the hepatic artery, as well as insertion of sampling catheters into the left common hepatic vein, the portal vein, and a femoral artery, and the insertion of infusion catheters into a splenic and a jejunal vein (9). Diet, housing, protocol approval, criteria for study, and preparation for study were as previously described (15).

Each experiment consisted of a 90-min equilibration period (−120 to −30 min), a 30-min basal period (−30 to 0 min), and a 240-min experimental period (0–240 min). At −120 min, priming doses of [U-^{14}C]glucose (11 μCi/kg) and [3-^{3}H]glucose (34 μCi/kg) were given, and constant infusions of [U-^{14}C]glucose (0.4 μCi/min), [3-^{3}H]glucose (0.35 μCi/min), and indocyanine green dye (0.14 mg/min) (Sigma, St. Louis, MO) were initiated. A constant peripheral infusion of p-aminohippuric acid (PAH) (1.7 μmol · kg^{-1} · min^{-1}) (Sigma) was also started at −120 min, continuing until 0 min. At 0 min, a constant peripheral infusion of somatostatin (0.8 μg · kg^{-1} · min^{-1}) (Bachem, Torrance, CA) was begun to suppress endogenous insulin and glucagon secretion, and porcine insulin (0.4 mU · kg^{-1} · min^{-1}) (Eli Lilly, Indianapolis, IN) and glucagon (0.6 ng · kg^{-1} · min^{-1}) (Novo Nordisk, Bagsvaerd, Denmark) were infused intraportally to maintain basal levels. Nicotinic acid (NA) (pH adjusted to 7.0) (Sigma) was infused peripherally at 1.5 mg · kg^{-1} · min^{-1}. A constant intraportal infusion of 20% dextrose (21.3 μmol · kg^{-1} · min^{-1}) (Baxter Healthcare, Deerfield, IL) mixed with PAH (1.7 μmol · kg^{-1} · min^{-1}) was also started at 0 min. In addition, a primed variable-rate peripheral infusion of 50% dextrose (Abbott) was begun at 0 min in each group to clamp blood glucose quickly at the desired value. After 2 h (0–120 min; period 1), the dogs were divided into three groups. In one group (NA plus lipid emulsion [NAL], n = 8) all of the infusions of period 1 continued, with the addition of peripheral venous infusions of Intralipid 20% fat emulsion (0.02 ml · kg^{-1} · min^{-1})

TABLE 1.
Hormone concentrations in 42-h–fasted conscious dogs receiving intraportal insulin and glucagon infusions at basal rates, intraportal glucose infusion, and peripheral infusions of NA, NAG, or NAL

Parameter and group	Study period		
	Basal	Period 1	Period 2
Arterial plasma insulin (pmol/l)			
NA	50 ± 10	34 ± 6	37 ± 6
NAG	46 ± 28	36 ± 14	43 ± 17
NAL	47 ± 10	32 ± 7	34 ± 8
Hepatic sinusoidal plasma insulin (pmol/l)			
NA	141 ± 26	137 ± 12	149 ± 22
NAG	109 ± 65	131 ± 34	148 ± 31
NAL	147 ± 43	127 ± 20	151 ± 28
Arterial plasma glucagon (ng/l)			
NA	38 ± 4	37 ± 4	34 ± 4
NAG	30 ± 2	33 ± 3	30 ± 3
NAL	44 ± 4	35 ± 5	33 ± 4
Hepatic sinusoidal plasma glucagon (ng/l)			
NA	42 ± 5	51 ± 4	47 ± 4
NAG	34 ± 4	51 ± 4	45 ± 4
NAL	51 ± 4	53 ± 5	47 ± 5
Arterial plasma cortisol (nmol/l)			
NA	93 ± 12	121 ± 14	67 ± 7
NAG	104 ± 26	103 ± 35	70 ± 4
NAL	139 ± 31	99 ± 16	60 ± 8

Data are means $\pm$ SE of two sampling points during the basal period and 5 sampling points during periods 1 and 2. There were no significant differences among groups ($n = 7$, 3, and 8 for NA, NAG, and NAL, respectively).

(Fresenius Kabi Clayton, Clayton, NC) and heparin ($0.5 \, \text{units} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$, to stimulate lipoprotein lipase activity) (Elkins-Sinn, Cherry Hill, NJ) for the remainder of the experimental period (120–240 min; period 2). A second group received a peripheral infusion of glycerol (NA plus glycerol [NAG], $n = 3$) (Fisher Scientific, Fair Lawn, NJ) at $0.65 \, \text{mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ during period 2 to create circulating glycerol concentrations equivalent to those in the NAL group, and the third group (NA, $n = 7$) received saline rather than the lipid emulsion/heparin or glycerol infusions during period 2.

Femoral artery, portal vein, and hepatic vein blood samples were taken every 15–30 min during the basal (-30 to 0 min) and experimental (0–240 min) periods, and a hyperglycemic clamp was performed, as previously described (23). After completion of each experiment, the animal was sedated with pentobarbital and liver biopsies were taken before killing (23).

Processing and analysis of samples. Hematocrit, plasma glucose, blood glucose, lactate, alanine, glycerol, β-hydroxybutyrate (βOHB), acetoacetate, $^{14}CO_2$, as well as plasma glucose, NEFA, insulin, and glucagon concentrations, and ^3H and ^{14}C glucose, were determined as described previously (9,23,24). Hepatic glycogen (25) and glucose 6-phosphate (G6P) (26) concentrations were also determined.

Calculations and data analysis. Hepatic blood flow measurements with ultrasonic flow probes and indocyanine green dye were not significantly different, but the data reported here use ultrasonic-determined flows (23).

The recovery of PAH across the liver was measured to assess mixing of the infusate with the portal blood and utilized as a criterion for inclusion of the experiment in the database (23). In the 18 animals included in the database, the ratio of recovered to infused PAH in the portal and hepatic veins was 0.9 ± 0.0 and 0.8 ± 0.0, respectively, with a ratio of 1.0 representing ideal mixing.

The hepatic substrate load, net hepatic balance, net fractional hepatic extraction, nonhepatic glucose uptake, hepatic glucose oxidation, and hepatic sinusoidal insulin and glucagon concentrations were calculated as described previously, using both direct and indirect calculations for glucose balance (24). Glucose balance data reported here use the direct calculation unless stated otherwise. The $[^3H]$ hepatic glucose balance was divided by the weighted (for the proportion of flow contributed by the hepatic artery and portal vein) inflowing plasma $[^3H]$ glucose specific activity (dpm/μmol glucose) to yield the unidirectional HGU. Unidirectional hepatic glucose release (HGR) was the difference between NHGU and HGU. The rates of glucose appearance (R_a) and disappearance (R_d) were calculated with a two-compartment model using dog parameters (27,28). Endogenous R_a was calculated as R_a − (peripheral glucose infusion rate + [portal glucose infusion rate $\times$ 1 − net hepatic fractional glucose extraction]).

Net carbon retention by the liver, representing glucose storage as glycogen in the liver, was NHGU + (net hepatic alanine uptake + net hepatic glycerol uptake) − (net hepatic lactate output + net hepatic CO_2 production), with all values in glucose equivalents. This calculation omits the contribution of gluconeogenic amino acids other than alanine, but the total of their net hepatic uptakes is no more than that of alanine ($\sim 1.5 \, \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ glucose equivalents) under a variety of hyperglycemic conditions (9,29,30). Net (or "measured") hepatic glycogen synthesis was the difference between hepatic glycogen concentrations at the end of experimentation and those in 11 42-h–fasted dogs that did not undergo experimentation (15). The maximum rate of gluconeogenic flux was calculated from the net hepatic uptake of gluconeogenic precursors, assuming 100% conversion to glucose (31).

Statistical analysis. Data are means $\pm$ SE. Statistical analysis was performed by ANOVA and ANOVA for repeated measures, with post hoc analysis by the Student-Newman-Keuls procedure (SigmaStat; SPSS, Chicago, IL). Statistical significance was accepted at $P < 0.05$. All data described in RESULTS for periods 1 and 2 are the mean of the all sampling times in each period, unless otherwise specified.

RESULTS

Plasma hormone concentrations. The arterial and hepatic sinusoidal insulin and glucagon concentrations and the arterial cortisol concentrations remained basal and indistinguishable among the groups (Table 1).

Arterial plasma levels and net hepatic uptake of NEFA. The arterial plasma NEFA concentrations declined similarly in all groups during period 1 (Fig. 1). The NEFA concentration in the NA and NAG groups continued to decline slightly during period 2, reaching values $\leq 10\%$ of basal. In the NAL group, the NEFA concentration returned to basal during period 2, averaging $914 \pm 57 \, \mu\text{mol/l}$ ($P < 0.05$ vs. the other groups). Net hepatic NEFA uptake decreased to near zero in all groups during period 1 ($P = 0.2$ among groups). Net hepatic NEFA uptake during period 2 averaged 0.1 ± 0.1 and $0.1 \pm 0.0 \, \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ in the NA and NAG groups, respectively, while it increased to $1.8 \pm 0.2 \, \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ in the NAL group ($P < 0.05$ vs. the other groups; NS versus basal).
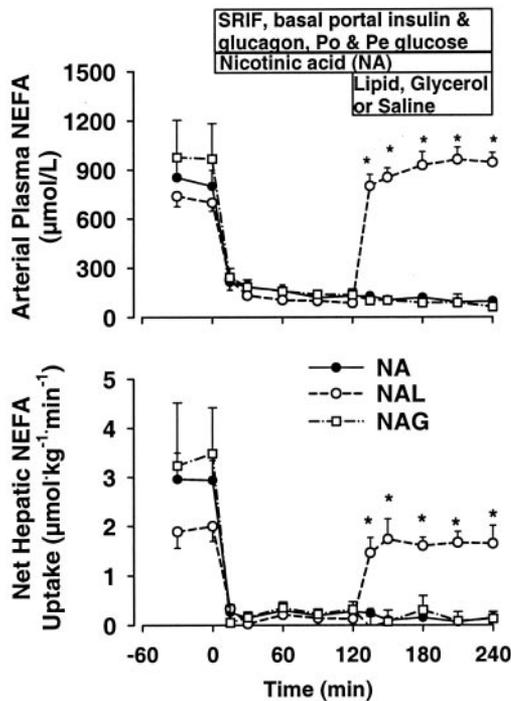
FIG. 1. Arterial plasma NEFA and net hepatic NEFA balance in 42-h–fasted conscious dogs receiving intraportal (Po) insulin and glucagon infusions at basal rates, glucose infusions, and peripheral (Pe) infusions of NA ($n = 7$), NAG ($n = 3$), or NAL and heparin ($n = 8$). *$P < 0.05$ vs. the other two groups. SRIF, somatostatin.
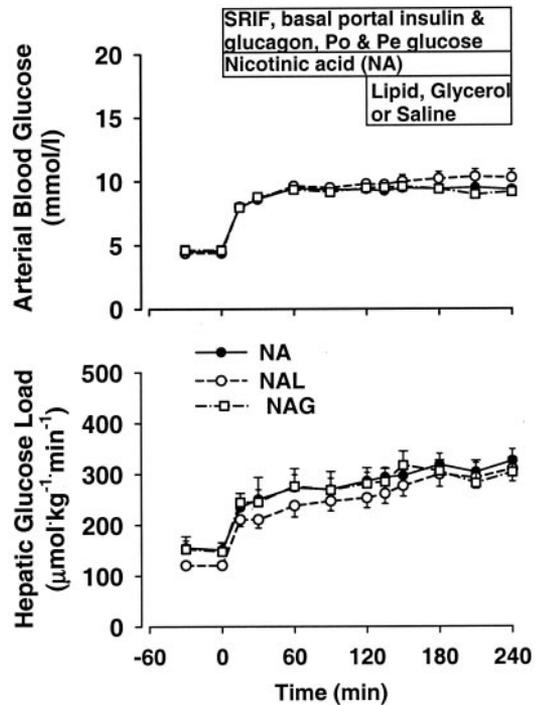

FIG. 2. Arterial and portal blood glucose concentrations and hepatic glucose load in 42-h–fasted conscious dogs receiving intraportal (Po) insulin and glucagon infusions at basal rates, glucose infusions, and peripheral (Pe) infusions of NA ($n = 7$), NAG ($n = 3$), or NAL and heparin ($n = 8$). There were no differences among the groups. SRIF, somatostatin.

Blood glucose concentrations, hepatic blood flow, and hepatic glucose load. The arterial and portal vein blood glucose levels increased twofold during the experimental period and were not different among the groups (Fig. 2), with a similar arterial-portal blood glucose gradient (~0.8 mmol/l) in all groups. Likewise, the hepatic blood flow was very similar among the groups during both the basal period (35 ± 3, 33 ± 5, and 30 ± 2 ml · kg^{-1} · min^{-1} in the NA, NAG, and NAL groups, respectively) and the experimental periods (28 ± 2, 27 ± 4, and 25 ± 2 ml · kg^{-1} · min^{-1}, respectively, in period 1 and 31 ± 2, 30 ± 3, and 29 ± 2 ml · kg^{-1} · min^{-1}, respectively, in period 2; data not shown).

The hepatic glucose load doubled during the glucose infusion period and did not differ significantly among the groups (154 ± 13 [basal] to 263 ± 21 and 307 ± 20 during periods 1 and 2, respectively, in the NA group; 152 ± 23 to 262 ± 31 and 299 ± 25 in the NAG group; and 129 ± 10 to 241 ± 18 and 299 ± 22 μmol · kg^{-1} · min^{-1} in the NAL group). The tendency of the hepatic glucose load to rise over time in all groups resulted primarily from the slight increase in hepatic blood flow during period 2, apparently related to the use of NA. Nevertheless, there were no significant differences in hepatic glucose load among groups.

Net hepatic glucose balance, net hepatic fractional glucose extraction, glucose infusion rates, nonhepatic glucose uptake, and glucose turnover. Net hepatic glucose output (~8–10 μmol · kg^{-1} · min^{-1}) was similar during the basal period in each group. Coincident with the start of period 1, the livers switched from net output to net uptake, with the rate during period 1 averaging 6.9 ± 1.6, 9.5 ± 5.2, and 6.3 ± 1.7 μmol · kg^{-1} ·

min^{-1} in the NA, NAG, and NAL groups, respectively (Fig. 3) ($P = 0.6$ among groups). The mean rates of NHGU during period 2 were 10.5 ± 2.0, 12.8 ± 0.9, and 4.7 ± 1.9
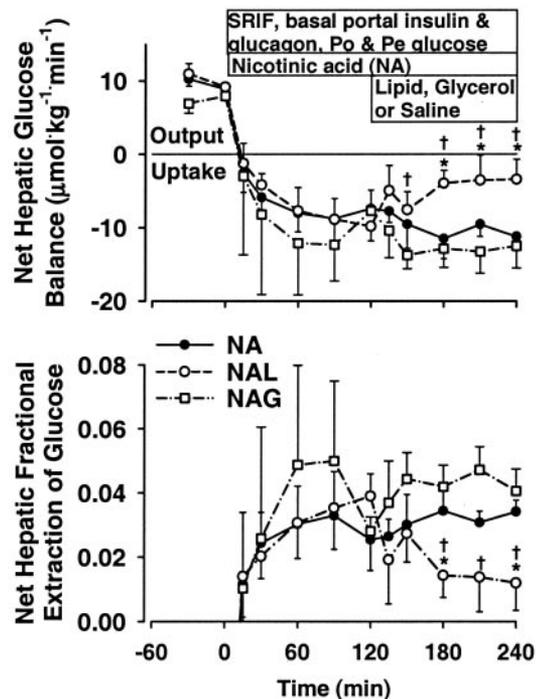

FIG. 3. Net hepatic glucose balance and net hepatic fractional glucose extraction in 42-h–fasted conscious dogs receiving intraportal (Po) insulin and glucagon infusions at basal rates, glucose infusions, and peripheral (Pe) infusions of NA ($n = 7$), NAG ($n = 3$), or NAL and heparin ($n = 8$). *$P < 0.05$ vs. NA group; †$P < 0.05$ vs. NAG group. SRIF, somatostatin.
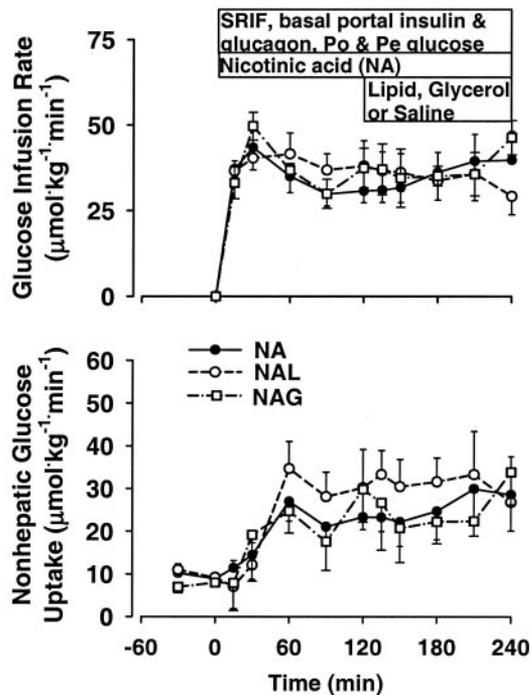
FIG. 4. Total glucose infusion rates and nonhepatic glucose uptake in 42-h–fasted conscious dogs receiving intraportal (Po) insulin and glucagon infusions at basal rates, glucose infusions, and peripheral (Pe) infusions of NA ($n = 7$), NAG ($n = 3$), or NAL and heparin ($n = 8$). There were no significant differences among the groups. SRIF, somatostatin.
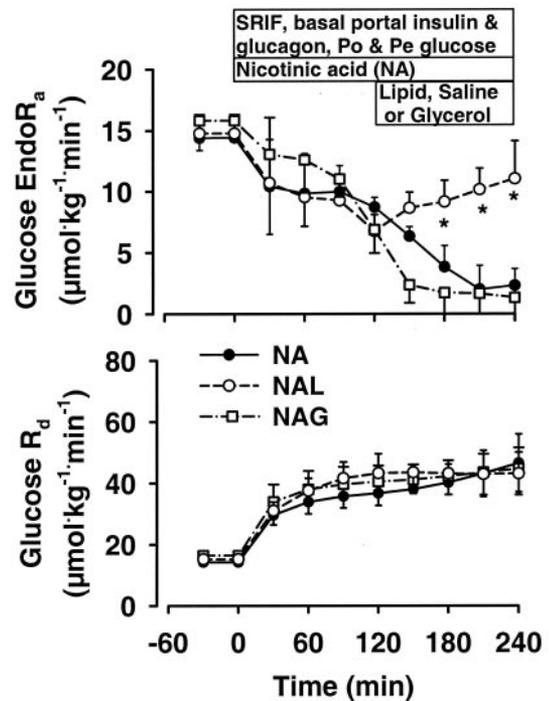

FIG. 5. Endogenous R_a and R_d in 42-h–fasted conscious dogs receiving intraportal (Po) insulin and glucagon infusions at basal rates, glucose infusions, and peripheral (Pe) infusions of NA ($n = 7$), NAG ($n = 3$), or NAL and heparin ($n = 8$). *$P < 0.05$ vs. the other two groups. SRIF, somatostatin.

μmol $\cdot$ kg^{-1} $\cdot$ min^{-1} in the NA, NAG, and NAL groups, respectively ($P < 0.05$ for NAL vs. the other two groups). The rates obtained using the indirect calculation were not significantly different from those with the direct calculation (13.3 $\pm$ 3.3, 15.0 $\pm$ 6.3, and 5.1 $\pm$ 3.2 μmol $\cdot$ kg^{-1} $\cdot$ min^{-1}, respectively). The net hepatic fractional extraction of glucose during period 1 did not differ among the groups ($P = 0.6$); during period 2, it averaged 0.031 $\pm$ 0.005, 0.043 $\pm$ 0.004, and 0.017 $\pm$ 0.007 μmol $\cdot$ kg^{-1} $\cdot$ min^{-1} in the NA, NAG, and NAL groups, respectively ($P < 0.05$ for NAL vs. the other groups).

The mean rates of glucose infusion (portal plus peripheral) in the three groups did not differ significantly during either periods 1 or 2. In period 1, the rates averaged 31.4 $\pm$ 3.6, 35.1 $\pm$ 2.7, and 38.4 $\pm$ 5.4 μmol $\cdot$ kg^{-1} $\cdot$ min^{-1} in the NA, NAG, and NAL groups, respectively (Fig. 4). The rates in the NA and NAG groups increased slightly during period 2 (to 38.8 $\pm$ 7.2 and 38.2 $\pm$ 3.1 μmol $\cdot$ kg^{-1} $\cdot$ min^{-1}, respectively, $P < 0.05$ vs. period 1 in each group), but there was a tendency ($P = 0.06$) for exogenous glucose requirements to decrease in the NAL group (33.7 $\pm$ 6.6 μmol $\cdot$ kg^{-1} $\cdot$ min^{-1}). Nonhepatic glucose uptake did not differ significantly among the groups during either period (Fig. 4).

Glucose endogenous R_a declined similarly during period 1 in all groups (Fig. 5). In the NA and NAG groups, it continued to fall during period 2, reaching 2.3 $\pm$ 1.4 and 1.3 $\pm$ 4.1 μmol $\cdot$ kg^{-1} $\cdot$ min^{-1}, respectively, by the last sampling point ($P = 0.6$ between groups). In the NAL group, however, endogenous R_a increased during period 2, and the rate at the last time point (10.6 $\pm$ 1.0 μmol $\cdot$ kg^{-1} $\cdot$ min^{-1}, $P < 0.05$ vs. the NA group) was not different from

basal. Glucose R_d did not differ among the groups at any time ($P = 0.7$).

Tracer-determined HGU was very low and not significantly different among the groups during the basal period (Table 2), and it increased significantly in all groups during period 1. The rate of HGU remained stable in the NA group but tended to rise in the NAG group during period 2 ($P = 0.6$) and to fall in the NAL group ($P = 0.2$). As a consequence, HGU during period 2 tended to be lower in the NAL group than in the NA ($P = 0.09$) or NAG group ($P = 0.15$). HGR declined significantly ($\sim$80–90%) in all groups during period 1. HGR in the NA and NAG groups continued to fall during period 2, but it showed a tendency to rise during period 2 in the NAL group ($P = 0.09$ for period 2 vs. period 1 in the NAL group, $P = 0.09$ for the NAL vs. NA group, and $P = 0.14$ for the NAL vs. NAG group).

Glycerol metabolism. In all groups, arterial blood glycerol levels declined 60–70% during period 1 (Table 2). The concentrations were stable in the NA group during period 2, but they increased approximately sixfold ($P < 0.05$ vs. the NA group) in the NAG and NAL groups. Net hepatic glycerol uptake fell in all groups to rates that were only 10–15% of basal during period 1. The rate of net hepatic glycerol uptake did not change in the NA group after that time, but during period 2 it increased 7-fold in the NAG group and 10-fold in the NAL group ($P < 0.05$ for both groups vs. the NA group, $P = 0.7$ for the NAG vs. NAL group).

Lactate and alanine metabolism. The arterial blood lactate concentrations were not significantly different among the groups at any time. The livers in all groups exhibited net hepatic lactate uptake during the basal period ($\sim$8 μmol $\cdot$ kg^{-1} $\cdot$ min^{-1}) (Fig. 6). After the

TABLE 2
Tracer-determined HGU and HGR, net hepatic CO_2 production, and arterial blood concentrations of substrates and ketones in 42-h–fasted conscious dogs receiving intraportal insulin and glucagon infusions at basal rates, intraportal glucose infusion, and peripheral infusions of NA, NAG, or NAL

Parameter and group	Study period		
	Basal	Period 1	Period 2
HGU ($\mu mol \cdot kg^{-1} \cdot min^{-1}$)			
NA	0.0 ± 1.1	9.8 ± 1.8	9.7 ± 1.3
NAG	0.8 ± 0.7	8.9 ± 4.2	11.3 ± 6.6
NAL	1.0 ± 1.2	8.4 ± 1.2	7.1 ± 1.5
HGR ($\mu mol \cdot kg^{-1} \cdot min^{-1}$)			
NA	9.5 ± 1.5	2.0 ± 2.1	-0.8 ± 2.2
NAG	8.3 ± 2.7	0.9 ± 1.7	-1.6 ± 4.7
NAL	11.0 ± 1.0	1.6 ± 2.0	2.7 ± 2.1
Net hepatic CO_2 production (μmol glucose equivalents $\cdot kg^{-1} \cdot min^{-1}$)			
NA	0.3 ± 0.2	0.7 ± 0.2	1.0 ± 0.2
NAG	0.4 ± 0.4	0.7 ± 1.9	0.8 ± 2.4
NAL	0.2 ± 0.1	1.0 ± 0.1	$0.5 \pm 0.1*$
Arterial blood glycerol ($\mu mol/l$)			
NA	89 ± 8	28 ± 2	24 ± 4
NAG	100 ± 20	27 ± 4	$163 \pm 17*$
NAL	93 ± 9	34 ± 3	$196 \pm 11*$
Net hepatic glycerol uptake ($\mu mol \cdot kg^{-1} \cdot min^{-1}$)			
NA	2.0 ± 0.4	0.2 ± 0.1	0.2 ± 0.1
NAG	2.8 ± 0.9	0.4 ± 0.1	$2.9 \pm 0.1*$
NAL	1.9 ± 0.3	0.3 ± 0.0	$3.6 \pm 0.2*$
Arterial blood alanine ($\mu mol/l$)			
NA	306 ± 34	491 ± 32	470 ± 45
NAG	229 ± 34	348 ± 38	335 ± 50
NAL	287 ± 18	402 ± 32	$353 \pm 33*$
Net hepatic alanine uptake ($\mu mol \cdot kg^{-1} \cdot min^{-1}$)			
NA	3.2 ± 0.7	2.8 ± 0.3	3.2 ± 0.3
NAG	2.0 ± 0.5	2.1 ± 0.6	2.2 ± 0.2
NAL	3.0 ± 0.3	2.7 ± 0.2	3.1 ± 0.2
Arterial blood acetoacetate ($\mu mol/l$)			
NA	83 ± 14	73 ± 7	84 ± 19
NAG	128 ± 27	113 ± 25	141 ± 30
NAL	97 ± 15	85 ± 14	99 ± 15
Net hepatic acetoacetate output ($\mu mol \cdot kg^{-1} \cdot min^{-1}$)			
NA	0.8 ± 0.6	0.2 ± 0.1	0.1 ± 0.3
NAG	2.2 ± 1.0	0.5 ± 0.6	0.4 ± 0.2
NAL	1.4 ± 0.5	0.3 ± 0.1	0.4 ± 0.2
Arterial blood βOHB ($\mu mol/l$)			
NA	38 ± 12	8 ± 1	5 ± 1
NAG	43 ± 17	$16 \pm 2*$	$13 \pm 1*$
NAL	39 ± 7	12 ± 3	$18 \pm 4*$
Net hepatic βOHB output ($\mu mol \cdot kg^{-1} \cdot min^{-1}$)			
NA	1.8 ± 0.6	0.2 ± 0.1	0.1 ± 0.0
NAG	1.3 ± 0.6	0.2 ± 0.0	0.1 ± 0.0
NAL	1.5 ± 0.3	0.2 ± 0.0	$0.4 \pm 0.0*†$

Data are means $\pm$ SE of two sampling points during the basal period and five sampling points during periods 1 and 2 ($n = 7, 3,$ and 8 for NA, NAG, and NAL, respectively). $*P < 0.05$ vs. NA; $†P < 0.05$ vs. NAG.

experimental period began, net hepatic lactate balance changed from uptake to output. In all groups, net hepatic lactate production peaked within 60 min. The NA and NAG groups continued to exhibit net hepatic lactate output throughout period 2 (mean rates 6.5 ± 1.7 and 4.9 ± 1.1 $\mu mol \cdot kg^{-1} \cdot min^{-1}$, respectively, $P = 0.6$ between groups), whereas the NAL group returned to net hepatic lactate uptake by 150 min, with uptake averaging 2.3 ± 1.2 $\mu mol \cdot kg^{-1} \cdot min^{-1}$ during period 2 ($P < 0.05$ vs. the other groups).

Arterial blood alanine concentrations increased 40–60% during period 1 in all groups (Table 2). The concentrations remained stable during period 2 in the NA and NAG groups, but fell 12% in the NAL group ($P < 0.01$ for period 2 vs. period 1 within the NAL group, $P < 0.05$ vs. the NA group). Net hepatic alanine uptake did not change significantly over time in any group.

Net hepatic $^{14}CO_2$ production, hepatic glycogen synthesis, hepatic G6P, and gluconeogenic flux. Net hepatic CO_2 production was minimal during the basal period (~ 0.3 μmol glucose equivalents $\cdot kg^{-1} \cdot min^{-1}$) (Table 2). During period 1, it increased significantly in all groups. The NA and NAG groups exhibited no significant change during period 2, but the rate decreased significantly in the
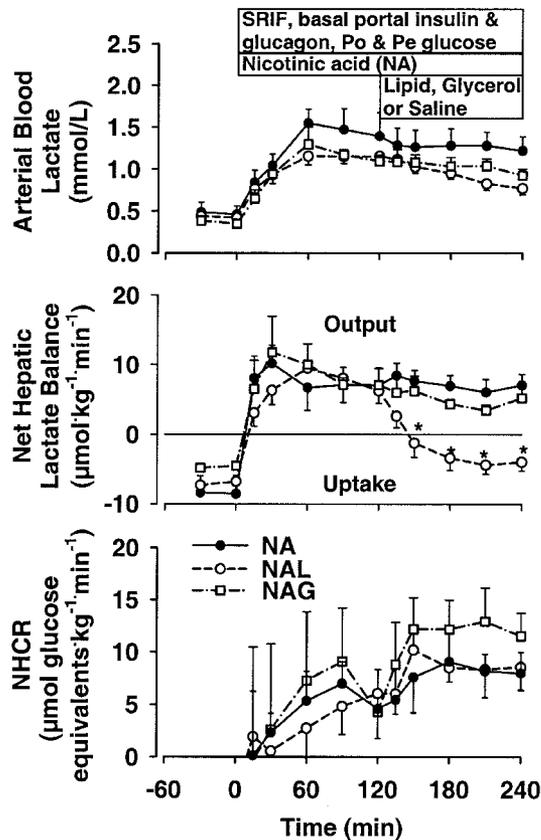
FIG. 6. Arterial blood lactate, net hepatic lactate balance, and NHCR in 42-h–fasted conscious dogs receiving intraportal (Po) insulin and glucagon infusions at basal rates, glucose infusions, and peripheral (Pe) infusions of NA (*n* = 7), NAG (*n* = 3), or NAL and heparin (*n* = 8). *$P < 0.05$ vs. the other two groups. SRIF, somatostatin.

NAL group during period 2, averaging only ~54% of that evident in the NA group ($P < 0.05$).

Measured net hepatic glycogen synthesis, averaged over the infusion period, was 6.1 ± 2.1, 12.2 ± 3.9, and 5.1 ± 2.9 μmol glucose equivalents $\cdot$ kg^{-1} $\cdot$ min^{-1} in the NA, NAG, and NAL groups, respectively (NS among groups, $P = 0.05$ for the NAG versus NAL group). Net hepatic carbon retention (NHCR) (Fig. 6), an indicator of the mass of carbon deposited as glycogen, did not differ significantly among groups at any time. NHCR during period 1 averaged 4.2 ± 1.5, 5.2 ± 4.4, and 3.0 ± 2.0 μmol glucose equivalents $\cdot$ kg^{-1} $\cdot$ min^{-1} in the NA, NAG, and NAL groups, respectively. NHCR during period 2 was 8.3 ± 2.1, 11.9 ± 1.1, and 8.6 ± 1.7 μmol glucose equivalents $\cdot$ kg^{-1} $\cdot$ min^{-1}, respectively. In a net sense, all of the glycogen synthesized in the livers of the NA and NAG groups could have been deposited via the direct pathway, i.e., directly from glucose taken up by the liver as opposed to glucose produced from gluconeogenic substrates in the liver. In the NAL group, all of the net hepatic glycogen synthesis calculated from NHCR could have occurred via the direct pathway during period 1, but at least 8–45% (depending on whether measured net synthesis or NHCR is used) must have been deposited via the indirect pathway during period 2 ($P < 0.05$ vs. the NA group). Hepatic G6P concentrations in the NA, NAG, and NAL groups were 109 ± 8, 77 ± 6, and 148 ± 6 nmol/g liver, respectively ($P < 0.05$ for NAL vs. the other two groups, $P < 0.05$ for the NAG vs. NA group).

The calculated maximal gluconeogenic flux rate was similar among the groups during period 1 (3.1 ± 0.4, 2.3 ± 0.6, and 2.9 ± 0.2 μmol glucose equivalents $\cdot$ kg^{-1} $\cdot$ min^{-1} in the NA, NAG, and NAL groups, respectively). The rates in the NA and NAG groups did not differ during period 2 (3.3 ± 0.3 and 3.7 ± 0.2 μmol glucose equivalents $\cdot$ kg^{-1} $\cdot$ min^{-1}), but the rate in the NAL group increased (6.3 ± 0.6 μmol glucose equivalents $\cdot$ kg^{-1} $\cdot$ min^{-1}, $P < 0.05$ vs. the NA and NAG groups).

Ketone metabolism. Arterial blood levels of acetoacetate did not change significantly in any group during any study period and did not differ among groups (Table 2). Net hepatic release of acetoacetate fell significantly in all groups during period 1. There was a tendency for net hepatic acetoacetate output to be greater in the NAL and NAG groups than the NA group during period 2, but this did not reach statistical significance ($P = 0.3$). Arterial blood βOHB concentrations fell significantly in all groups during period 1. Arterial βOHB concentrations continued to decrease in the NA group during period 2 (Δ -2 ± 1 μmol/l, $P < 0.05$ vs. period 1), tended to decrease in the NAG group ($P = 0.08$), but increased by 6 ± 1 μmol/l in the NAL group ($P < 0.05$ vs. the NA group, $P < 0.05$ vs. period 1 in the NAL group). Similarly, during period 1, net hepatic βOHB output fell by 1.6 ± 0.6, 1.1 ± 0.6, and 1.3 ± 0.4 μmol $\cdot$ kg^{-1} $\cdot$ min^{-1} in the NA, NAG, and NAL groups, respectively ($P = 0.8$ among groups). Net hepatic βOHB output remained low in the NA and NAG groups during period 2, while the NAL group exhibited a small but significant increase ($P < 0.05$ vs. the NA and NAG group).

DISCUSSION

In the presence of basal insulin and glucagon concentrations and the lipolytic inhibitor NA, portal glucose infusion (with the hepatic glucose load clamped at twofold basal) caused a rapid switch from net hepatic glucose output to uptake, with the rate averaging 10.5 ± 2.0 μmol $\cdot$ kg^{-1} $\cdot$ min^{-1} during the last 2 h of study. In a group of dogs previously studied in an identical manner to the NA group except that no NA was administered, NHGU during the same time period averaged 10.6 ± 1.7 μmol $\cdot$ kg^{-1} $\cdot$ min^{-1} (24), not significantly different from the rate in the NA group. In that study (24), the nadir arterial NEFA concentrations (276 ± 55 μmol/l) and net hepatic NEFA uptake (0.8 ± 0.2 μmol $\cdot$ kg^{-1} $\cdot$ min^{-1}) were significantly higher than those evident in the NA group. Clearly, the significant but comparatively small difference in NEFA concentrations and net hepatic uptake between the NA group and the previously reported group that did not receive NA did not have a detectable impact upon NHGU. On the other hand, in the present study, when NEFA concentrations were restored to near-basal levels in the presence of NA, NHGU was reduced by ~5.8 μmol $\cdot$ kg^{-1} $\cdot$ min^{-1} (55%) in comparison to the rate in the NA group. Enhancement of HGR and suppression of HGU contributed approximately equally to the suppression of NHGU. Endogenous glucose R_a was significantly enhanced during lipid infusion (difference between groups of ~6.3 μmol $\cdot$ kg^{-1} $\cdot$ min^{-1}), sufficient to explain the difference in NHGU between groups. Our data indicate that the difference in NEFA concentrations, rather than the increase in glycerol availability, was responsible for the differences in glucose R_a

and NHGU between the NA and NAL groups, since the elevation of glycerol in the presence of NA failed to impair NHGU or enhance glucose R_a.

The stimulation of HGR and glucose R_a during lipid infusion is consistent with previous in vitro and in vivo data. In hepatocytes of both normal and diabetic rats, glucose cycling between glucose and G6P was elevated when glycolysis was suppressed by an increase in fatty acids in the media (32–34) and the elevation of glucose cycling was associated with an increased flux through glucose 6-phosphatase (33). Lipid infusion rapidly increased hepatic glucose 6-phosphatase gene expression and protein content in rats (35). Enhancement of splanchnic glucose production was also observed in nondiabetic humans receiving Intralipid during a hyperinsulinemic, hyperglycemic clamp, although endogenous glucose production was not enhanced (36). The difference in findings regarding endogenous glucose production between our current study and that of Shah et al. (36) may have occurred because of the use of hyperinsulinemic conditions in the human study, whereas our investigation was conducted at euinsulinemia. Certainly, there is evidence that elevation of NEFA can alter endogenous glucose production in humans. In healthy volunteers studied under euglycemic-euinsulinemic conditions, endogenous glucose production was significantly stimulated after 2.5 h Intralipid infusion compared with a control study in which glycerol was infused. When the glucose concentrations were subsequently elevated to 10 mmol/l with euinsulinemia maintained, endogenous glucose production fell ~74% during glycerol infusion but did not decrease significantly during lipid infusion (37).

Hepatic glucose oxidation was significantly greater during period 2 in the NA than in the NAL dogs and tended to be greater in the NAG than in the NAL group, as might be expected based on the difference in NHGU. In addition, glycolysis was enhanced in the NA and NAG groups versus the NAL group, as evidenced by continued net hepatic release of lactate throughout period 2, compared with a shift to net hepatic lactate uptake in the NAL group. In dogs fasted 18 h with a selective increase in peripheral insulin levels (from 36 to 120 pmol/l, with portal vein insulin concentrations remaining basal), net hepatic glucose output fell by 50% over 3 h, with a simultaneous rise in net hepatic lactate output (30). The increase in lactate output in the dogs with the selective rise in peripheral insulin levels paralleled a decline in arterial NEFA levels and net hepatic NEFA uptake. Subsequently, we determined that maintaining basal NEFA levels during a selective increase in peripheral insulin prevented the rise in net hepatic lactate output (9). Thus, it appears that the fall in the levels and net hepatic uptake of NEFA was responsible for directing intrahepatic carbon into glycolysis in vivo. Under usual circumstances, of course, NEFA concentrations fall in the presence of an increase in insulin concentrations. In the current investigation, the fall in NEFA concentrations was brought about without hyperinsulinemia. Nevertheless, our current and previous (38,39) data confirm that suppression of plasma NEFA concentrations shifts the liver into a more glycolytic mode. In vitro evidence has shown that glycolysis is inhibited in isolated hepatocytes incubated with fatty acids (32,40). The shift toward glycolysis when fatty acid levels are low could result from a fall in citrate, one of the major inhibitors of phosphofructokinase, the first rate-determining enzyme in the glycolytic pathway (32,40). Alternatively, the difference in net hepatic lactate output between the NA and NAL groups might be explained by a difference in the intrahepatic redox state. The hepatic vein acetoacetate-to-βOHB ratio did not differ significantly between periods 1 and 2 in the NA group (9.8 ± 3.2 and 7.2 ± 1.2, respectively, $P = 0.4$) and tended to rise in NAG (7.0 ± 2.1 and 11.4 ± 3.3, $P = 0.06$), suggesting that the NADH-to-NAD ratio remained relatively stable or actually increased in those groups. In contrast, in the NAL group the hepatic vein acetoacetate-to-βOHB ratio showed a more pronounced tendency to fall, with the ratios being 8.5 ± 3.4 and 3.7 ± 0.8 in periods 1 and 2, respectively, $P = 0.05$.

The hepatic conservation of carbon resulting from the shift away from hepatic lactate output in the NAL group, along with the increased net hepatic glycerol uptake and decreased net hepatic CO_2 release in that group, resulted in similar net hepatic carbon retention and glycogen synthesis rates among the groups. That G6P concentrations were greater in the NAL group than in the other groups may reflect expansion of the G6P pool associated with a reduction in glycolysis. Randle et al. (41) postulated that muscle glucose uptake is reduced in response to increased NEFA availability by a mechanism involving an increase in intracellular G6P, leading to a feedback inhibition of hexokinase II activity and consequent inhibition of glucose uptake. Based on our findings, a similar mechanism could operate in the liver, with the caveat that glucokinase, rather than hexokinase II, is the primary hepatic enzyme involved in glucose phosphorylation.

Interestingly, as mentioned above, net hepatic glycogen deposition (whether measured directly or indirectly via calculation of net hepatic carbon retention) did not differ significantly between the NA and NAL groups, and only the direct measurement differed between the NAG and NAL groups, despite a significant difference between the NAL group and the other groups in hepatic concentrations of G6P, an allosteric activator of glycogen synthase. In agreement with this, Gustafson et al. (33) observed that hepatocytes in a glucose medium exhibited no difference in the rate of glycogen synthesis in the presence and absence of oleate. Gustafson et al. (33) also noted that enhancement of hepatocyte G6P levels by inhibition of G6P translocase did not stimulate glucose synthase activity in either the presence or absence of oleate. Thus, changes in hepatic G6P do not always result in changes in glycogen synthase activity, a phenomenon that might be related to the metabolic zonation of the liver (42) or to compartmentation of G6P within the hepatocyte (43,44). Net hepatic glycogen synthesis is the sum of the processes of glycogen synthesis and glycogenolysis. We have previously shown that Intralipid infusion in the presence of euglycemia and basal insulinemia inhibited glycogenolysis ~35% (38). However, glycogenolysis appeared even more sensitive to hyperglycemia; a more modest elevation of blood glucose (6.7 mmol/l) than achieved in the current experiments reduced glycogenolysis ~56% in the absence of Intralipid infusion (38). Similar results have been obtained in individuals with type 1 diabetes (45). Thus, our failure to

observe any enhancement of net hepatic glycogen synthesis resulting from suppression of glycogenolysis in the NAL group may result from the fact that glycogenolysis was already substantially suppressed by hyperglycemia in all three groups.

The rate of gluconeogenic flux was significantly enhanced in the NAL group in comparison to the other groups during period 2. The difference in the flux rates was similar in magnitude to the difference in NHGU and HGR between the NAL group and the other two groups but could not explain all of the difference among groups in endogenous R_a. This is probably because endogenous R_a includes the contribution of the kidney, and in vitro data indicate that NEFAs stimulate renal gluconeogenesis (46).

In contrast to NHGU, neither total body glucose disposal (reflected in the glucose infusion rates and glucose R_d) nor nonhepatic glucose uptake was significantly affected by lipid infusion. Lipid infusion has been shown to reduce insulin-stimulated glucose uptake in skeletal muscle in rats (47,48) and humans (49,50). However, lipid infusion did not inhibit glucose uptake under euinsulinemic-euglycemic conditions in normal men (3) or under "basal" conditions in individuals with type 2 diabetes (with prevailing hyperglycemia of ~11.5 mmol/l and hyperinsulinemia of ~150 pmol/l) (50). Nevertheless, lipid administration did suppress total body glucose uptake in euglycemic (5 mmol/l) individuals with type 2 diabetes when insulin concentrations were clamped at 550 pmol/l and in hyperglycemic (~11.5 mmol/l) individuals with type 2 diabetes when insulin concentrations were raised to ~900 pmol/l (50). In addition, Intralipid infusion significantly reduced both leg glucose uptake and glucose R_d in normal subjects during a hyperinsulinemic-hyperglycemic clamp (36). Thus, the fact that our study was conducted under basal insulinemic conditions likely provides part of the explanation as to why there was no detectable suppression of total body or nonhepatic glucose uptake during lipid infusion. Additionally, Roden et al. (7) found that lipid infusion rapidly (within 45 min) inhibited skeletal muscle glucose transport and/or phosphorylation but inhibited whole-body glucose uptake only after 140 min. Suppression of glucose R_d was also observed under euglycemic-euinsulinemic conditions in normal men after 2.5 h of Intralipid infusion, although a reduction of intramuscular G6P was manifest much earlier (37). Since our focus was on the liver, we used a shorter infusion period that probably was not long enough to make defects in whole-body glucose disposal manifest.

In conclusion, maintaining arterial NEFA concentrations at near-basal concentrations in the presence of basal insulinemia, hyperglycemia, and portal glucose infusion significantly reduced NHGU. The reduction in NHGU was due, in approximately equal proportions, to a tendency to restrain hepatic glucose uptake and stimulate hepatic glucose release.

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